

## 1. DESCRIPTION

The organism responsible for causing *viral nervous necrosis* (VNN) or viral encephalopathy and retinopathy (VER) has been demonstrated to belong to the group *Nodaviridae* and is an icosahedral, non-enveloped virus, 25-30 nm in diameter. These agents are also known as Striped Jack Nervous Necrosis Virus (SJNNV) and Fish Encephalitis Virus (FEV). All share serological similarities with the exception of those affecting turbot. VNN occurs in Asia, the Mediterranean and the Pacific.

VNN affects the nervous system. All affected species show abnormal swimming behaviour (cork-screwing, whirling, darting and belly-up motion) accompanied by variable swim bladder hyperinflation, cessation of feeding, changes in colouration, and mortality. Differences between species are most apparent with relation to age of onset and clinical severity. Earlier clinical onset is associated with greater mortality, thus onset at one day post-hatch in striped jack results in more severe losses than suffered by turbot, where onset is up to three weeks post-hatch. Mortalities range from 10-100%. Two forms of VER have been induced with experimental challenges. Acute - induced by intramuscular inoculation. Sub-acute - by intraperitoneal inoculation, bath, cohabitation and oral routes.

VetPCR™ VNNV Detection Kit is the direct detection of *Viral nervous necrosis virus* on the basis of a genetic database, so it can diagnose very fast and accurately. It can amplify only specific gene using the PCR (Polymerase Chain Reaction) method, and take only 3 hours for detection. Therefore, it is a very fast, accurate and reliable technique.

## 2. STORAGE

The components of VetPCR™ VNNV Detection Kit should be stored at -20°C. Under this condition, the kit is stable until expiration date stated on the label.

## 3. CONTENTS

	Kit 48	Kit 96	
VetPCR™ VNNV RT-PCR Pre-mixture.....	48	96	tubes
VetPCR™ VNNV PCR Pre-mixture .....	48	96	vial
BrigRT-PCR™ solution .....	1	1	vial
Biotech™ Transcriptase solution .....	1	1	vial
DNase/RNase-free water .....	1	1	vial
VNNV RT-PCR Positive control .....	1	1	vial
VNNV RT-PCR Positive control Pre-mixture .....	4	8	tubes
VNNV PCR Positive control Pre-mixture .....	4	8	tubes
Brig™ Molecular Weight marker .....	1	1	vial
Mineral Oil .....	1	2	vial(s)
RNA extraction kit (see step 6.1) .....	50	100	tests

## 4. SPECIMEN

Tissue, ovas, sperm, feces.

## 5. ADDITIONAL REQUIRED MATERIALS

- Pipettes, Sterile pipette tip, Vortex mixer
- Centrifuge for microcentrifuge tubes
- Thermal cycler, Electrophoresis kit, UV transilluminator

## 6. PROCEDURE

Please read through the entire procedure before starting.

### 6.1 RNA PREPARATION

Various manufacturers offer RNA isolation kits. Please carry out the RNA isolation according to the manufacturers instructions. The following standard RNA Purification kit is recommended.

Product	Catalog No.	Manufacturer
Bioingentech™ Total RNA Purification Kit (50 test)	230041(50)	Bioingentech Biotechnology Inc.
Bioingentech™ Total RNA Purification Kit(100 test)	230041(100)	Bioingentech Biotechnology Inc.

### 6.2 AMPLIFICATION

1.- Prepare appropriate RT-PCR Premix tubes and one RT- PCR Premix tube for Positive control. Label.

2.- Add 5µl of DNase/RNase-free water into the RT-PCR Premix tube to total volume as 8,5µl.

3.- Add 1,5µl of template RNA into the RT-PCR Premix tube to total volume as 10µl.

4.- Add 5µl of DNase/RNase-free water and 1,5µl of RT-PCR Positive control into a RT-PCR Positive control Premix tube for monitoring of amplification and easy interpretation.

5.- Add mineral oil (11µl). This step is necessary, even when using a thermal cycler that employs a top heating method.

6.- Perform RT-PCR reaction (RT-PCR 1) of samples as the below process using a PCR thermal cycler.

7.- Add 0,3µl of BrigRT-PCR™ solution and 0,5µl of Biotech™ Transcriptase solution.

8.- Perform RT-PCR reaction (RT-PCR 2) of samples as the below process, using a PCR thermal cycler.

		RT-PCR cycle		Temp.	Time
RT-PCR 1	1Cycle	Initial Denaturation		80°C	10 min.
	1Cycle	Stop		4°C	5 min.
Add 0,3µl of BrigRT-PCR™ and 0,5µl of Biotech™ Transcriptase					
RT-PCR 2	1Cycle	Denaturation		80°C	10 min.
	1Cycle	Annealing		25°C	10 min.
	1Cycle	Extension		37°C	50 min.

9.- Prepare appropriate PCR Premix tubes and one PCR Premix tube for Positive control. Label.

10.- Add 6µl of DNase/RNase-free water into the PCR Premix tube to total volume as 11µl.

11.- Add 2µl of template (cDNA) into the PCR Premix tube to total volume as 13µl.

12.- Add 6µl of DNase/RNase-free water and 2µl of Positive control (Positive control tube from RT-PCR) into a PCR Positive control Premix tube for monitoring of amplification and easy interpretation.

13.- Add mineral oil (11µl). This step is necessary, even when using a thermal cycler that employs a top heating method.

14.- Perform PCR reaction of samples as the below process, using a PCR thermal cycler.

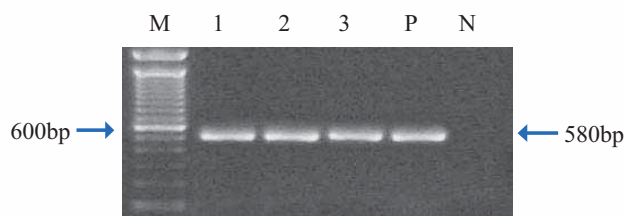
PCR cycle		Temp.	Time
1 Cycle	Initial Denaturation	94°C	2 min.
30 Cycles	Denaturation	94°C	30 sec.
	Annealing	54,5°C	30 sec.
	Extension	72°C	30 sec.
1 Cycle	Final extension	72°C	5 min.

### 6.3 DETECTION OF AMPLIFIED PRODUCTS

- 1.- Prepare 1.5% agarose gel containing Ethidium bromide (Et-Br).
- 2.- Load 7µl of PCR product, 7µl of Positive control and 2µl of Brig™ Molecular Weight marker on agarose gel without adding a loading-dye buffer and perform electrophoresis.
- 3.- Run electrophoresis by 100V (required about 30–40 minutes).
- 4.- Identify the result on ultra-violet (UV) transilluminator.

### 6.4 INTERPRETATION

- Expected PCR product size : 580 bp



**Fig 1.** Electrophoresis of PCR product by VetPCR™ VNNV Detection Kit  
 Lane M : Brig™ Molecular Weight Marker (Bioingentech Ltd.)  
 Lane 1~3 : VNNV Positive sample  
 Lane P : Positive control  
 Lane N : Negative control

### 7. NOTICE

- For research purpose only. Not for use in diagnostic procedures for clinical purposes. *For in Vitro Use Only.*
- Take care in handling of specimen to minimize risk of infection.
- The PCR process is covered by patents issued and applicable in certain countries. Bioingentech Biotechnology Inc. does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

### 8. TROUBLE SHOOTING

- 1.- In the case of difficult to interpret results due to non-specific bands; reduce amount of template by 1/10 dilution, heated at 65° C for 5 min. and reacts again.
- 2.- Preparation of PCR reaction at room temperature may cause the non-specific band.
- 3.- All procedure should be carried out on ice.

### 9. ORDERING INFORMATION

Product	Catalog No.
VetPCR™ VNNV Detection Kit 48	VET0006QR(48)
VetPCR™ VNNV Detection Kit 96	VET0006QR(96)
Brig™ Molecular Weight Marker	24012



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